

PROJECT NUMBER: 6912
PROJECT TITLE: Tobacco/Smoke Relationships
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I. TSNA PRECURSORS

- A. Objective: To determine the precursors of MS TSNA.
- B. Results: Thin layer radiochromatography (TLRC) of MS TPM extracts from radiolabeled nicotine cigarettes was continued. As described in the April monthly summary, the MS TPM buffer solutions were extracted with methylene chloride. Preparative chromatography of methylene chloride extracts was done on silica gel plates (1000 μ thickness) in methylene chloride/methanol (20/1). The LSC of bands obtained from preparative TLC of smoke samples showed that for smoking #1: of the radioactivity applied to the plates, 24% was associated with a broad region (zone 2) including an area co-chromatographed with NNK, and 53% with a more polar zone. Of the activity applied to the plate, ~77% was recovered. In smoking #2: of the radioactivity applied to the plates, ~80% was recovered. Of the recovered activity, 18% was associated with zone 2 (as described above), and 61% with a more polar zone.

Based on integration of the scans, it is estimated that 0.01 - 0.05% of the initial activity present in the cigarettes (50 μ Ci) may be associated with a peak that co-chromatographs with NNK. RP-HPLC in conjunction with radioactivity detection confirmed that zone 2 is a mixture of 14 C compounds, and that there is activity associated with retention times that corresponds to NNN/NNK (12.0 - 16.0 min.). (Reference mixtures of labelled TSNA and nicotine showed good resolution).

GC/TEA analysis of zone 2 from radiolabeled smoke samples showed NAT, NNN and NNK present. However, compared to the amounts determined for nonradiolabeled cigarettes, the loss of 70% of the expected TSNA delivery was indicated for the 14 C cigarettes. Some of this may be attributed to difficulties in smoking, collecting, and isolation methods.

- C. Plans: Analyze filler blend used for making cigarettes for nicotine and NNK content. Determine if study needs to be repeated, and refine smoke collection methods as needed.
- D. Reference:

1. Hassam, S. Notebook No. 8823, pp.

II. CROSSED SOLUBLES/BASE WEB STUDY (CHEMISTRY)

- A. Objective: To investigate the smoke chemistry of model cigarettes made from all possible combinations of solubles from bright, burley and oriental tobaccos on base webs from the three tobaccos.

- B. Results: Bright CEL with added nitrogen (in the form of amino acids, a protein or ammonium acetate) was sprayed on bright base web and made into cigarettes. In addition to the smokings of nitrogen addition samples reported last month, control sample smokings for the study were completed. The CSC from each smoking was submitted for testing in the S/M assay.

A sample of burley CEL insolubles was sprayed on to bright base web. The RL (Bu11/BrBW) was made into cigarettes, and attempts to smoke these cigarettes were successful.

Puff counts determined on calcium acetate addition cigarettes show that the puff count increases significantly from 10±1 to 13 when the amount of calcium added is ≥6% (total solids held constant).

Samples of burley CEL treated previously with various ion exchange resins were extracted with different solvents. Eight samples were produced. Based on sample dry weight the bulk of the CEL solubles is recovered in the neutrals. The dry sample from each extraction was dissolved in water. Of the eight samples produced six were sprayed on bright base web and made into model cigarettes.

- C. Plans: Preparation of fillers and cigarettes will be continued. Cigarettes will be smoked for the Salmonella/microsome assay. Formulation of plans for C Pilot Plant production of RL will be completed. Preliminary experiments to demineralize CEL using the electrodialysis unit will be continued.

D. References:

1. Hellams, R. Notebook No. 8613, pp. 163-164
2. Drew, S. Notebook No. 8899, pp. 29-37.

III. SUPPORT FUNCTION: CONDENSATE PREPARATION

- A. Objective: To fabricate cigarettes, perform smokings, and prepare condensate as needed for biological and chemical analysis.
- B. Results: Handmade cigarettes were prepared for Project 6908, 1902, 1620 and 6912. Cigarettes were smoked on a 30-port Borgwaldt smoking machine to collect CSC for S/M and EGF assay testing.
- C. Reference:
1. McGee, N. Notebook No. 8743, pp. 47-63.